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Degradation of Carmustine in Aqueous Media

PAUL A. LASKAR * and JAMES W. AYRES

Abstract □ The degradation rate of carmustine was investigated in buffered aqueous media at several pH values. The buffering agents studied were those with potential use in parenteral formulations of this drug: acetate, citrate, and phosphate. The apparent first-order degradation rate constants were calculated using a linear regression procedure. A pH range over which minimum degradation occurred was ascertained. General acid and specific base catalysis was demonstrated for the degradation of carmustine. From the data at 5, 22, and 37°, the apparent activation energies for carmustine degradation in buffered aqueous media were computed and were strongly pH dependent.

Keyphrases □ Carmustine—degradation rate in aqueous media, effect of pH and temperature □ Degradation rate—carmustine in aqueous media, effect of pH and temperature □ Antineoplastic agents—carmustine, degradation rate in aqueous media, effect of pH and temperature □ Stability—carmustine in aqueous media, effect of pH and temperature

Drugs for the treatment of neoplastic disease have received a great deal of attention. Several thousand chemical agents are systematically screened each year for antineoplastic activity, but only a small percentage exhibit activity and are tested further for clinical utility. Carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea; NSC 409962] (I) was effective in treating L-1210 leukemia, including cases where the central nervous system was involved (1–4).

Prior to the interest in I as a therapeutic agent, a study indicated that 1-methyl-1-nitrosourea (NSC 23909) (II) was unstable in aqueous solution (5), as was a nitrosamide-containing antibiotic, streptozocin, (6). The kinetics of I degradation were considered, and this compound also was unstable in aqueous media (4, 7).

Because I is often administered *via* intravenous solution (8, 9), further consideration of its stability in various aqueous media suitable for intravenous use is desirable. In addition, knowledge of the temperature dependence of the degradation of I is needed to obtain useful shelflife data and to determine desirable storage criteria. Furthermore, knowledge of acid or base catalysis also may be of value in formulating I.

The few published studies directed toward optimizing a parenteral formulation for I emphasized technological considerations such as lyophilization of drug solutions and dry filling with sterile solids (10–12). A formulation for clinical use was prepared by dry filling vials with sterile solid I and sterile, screened mannitol (11, 12). Alternatively, a solution of I in absolute ethanol was filtered through a 0.25-μm membrane and lyophilized (10, 11). These formulations should be used within 1 and 2 hr, respectively, following reconstitution with ethanol and dilution with saline (10, 12).

The purpose of this investigation was to perform accelerated stability studies on I in various aqueous media suitable for injection.

EXPERIMENTAL

Reagents—Carmustine, an investigational new drug, was obtained[1] as a lyophilized powder. All other chemicals were reagent grade.

Materials and Methods—All buffers and other solutions were prepared in volumetric flasks. Distilled water used for dissolving the basic component of the buffer systems was freshly boiled and cooled. Unless noted otherwise, solutions were stored at ambient temperature. Compound I, 0.025 g (stored at −19 ± 1°), was dissolved in sufficient cold (0°) ethanol to make 25 ml of stock solution. The stock solutions were stored at −19 ± 1° for not longer than 7 days.

Buffer solutions were prepared using published procedures (13, 14). All buffer solutions were checked for conformance to the desired pH using a pH meter calibrated at ambient temperature (20–22°) with a pH standard. When necessary, the pH was adjusted to within 0.05 pH unit of the nominal value with either the acidic or basic component of the buffer.

Degradation Experiments—All solvent systems were preequilibrated to the experiment temperature. For ambient (22 ± 1°) and physiological (37.0 ± 0.5°, forced-air oven) temperature experiments, the media were first allowed to equilibrate for at least 16 hr. For experiments at reduced temperature, the media were first chilled in a ice bath (1.0 ± 0.5°) for at least 15 min.

To a volume (~40–45 ml) of preequilibrated media was added 1.0 ml of I stock solution (1 μg/ml). Then the volume was brought rapidly to 50 ml with the appropriate medium, the mixture was shaken, and the time was recorded. A zero-time sample (0.2 ml) was taken for analysis, and the experimental solution was stored at the appropriate temperature. At designated time intervals, a sample volume (0.2, 0.5, 1.0, or 2.0 ml) was removed for analysis.

The criterion used to determine the sample size was that the apparent concentration should not fall below 1.0 μg/ml or exceed 5.0 μg/ml; the optimum range was 2–4 μg/ml to be well within the linear portion of the calibration curve. The sampling time interval depended in part on a previously observed or expected degradation rate.

Analytical Method—The colorimetric method of Loo and Dion (15) was utilized. The use of any substance that might interfere with the analytical method was scrupulously avoided.

An experimental sample volume not greater than 2.0 ml or estimated to contain between 1 and 5 μg of I/ml was added to a 17 × 150-mm Pyrex test tube. If necessary, sufficient distilled water was added to yield a total

[1] Merck Chemical Co. (through the National Cancer Institute).

Table I—Parameter Values for Linear Regressions of Logarithm of Apparent Degradation Rate Constants for I on pH

pH Condition	Temperature (°K)	Regression Coefficient (*SE*)	Intercept
Citrate buffer (pH 3.0, 4.0, and 5.0) and acetate buffer (pH 3.6, 4.0, and 5.0)	278	−0.106 (0.026)	0.001
Citrate buffer (pH 6.0); phosphate buffer (pH 6.0, 6.6, and 7.6); barbital buffer (pH 7.4 and 8.0)	295	1.27 (0.07)	8.86×10^{-6}
Phosphate buffer (pH 6.0, 6.6, and 7.6)	278	1.01 (0.04)	2.91×10^{-6}
	295	1.07 (0.05)	3.26×10^{-5}
	310	0.93 (0.13)	5.46×10^{-4}

volume of 2.0 ml and the contents were mixed. To this solution was added 1.0 ml of 0.5% (w/v) sulfanilamide in 2 *N* HCl, and the solution was mixed and incubated in a water bath with a shaker at 50 ± 0.01° for 45 min. The position in the water bath was randomized to minimize any position-dependent effects on the analytical results.

After 45 min, the solution in the tubes was rapidly chilled in an ice–water mixture. To the cooled solution was added 0.2 ml of 0.3% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride, and the mixture was agitated. The color was allowed to develop for 10 min, after which the percent transmittance was determined using a spectrophotometer[2] at 540 nm calibrated with a reagent blank. The order of percent transmittance determination of samples was randomized, and the amount of I remaining was determined with a calibration curve.

An initial experiment to determine the reliability of the standard curve used the following nominal concentrations prepared from the I stock solutions: 0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, and 50.0 μg/ml. The conformance of the colorimeter as well as the reagents to the calibration curve was checked at periodic intervals throughout the study using an abbreviated set of standards.

RESULTS AND DISCUSSION

The colorimetric method of Loo and Dion (15) was utilized to quantify I, and the calibration curve generated from pooled data was linear from about 0.5 to 7 μg/ml. When subsequent batches of reagent solutions were used, neither batch- nor time-dependent variation was detectable.

The effect of solvents and other adjuvants on drug degradation under some constant conditions can often be described according to the apparent first power of the drug concentration (16). A nitroso-containing compound related to the nitrosoureas, as well as several nitrosoureas, demonstrated first-order rate dependency (4, 6, 7, 17, 18). Therefore, the apparent first-order degradation rate constants for I in several buffer systems at three temperatures were computed from a linear regression of the logarithm of I remaining on time.

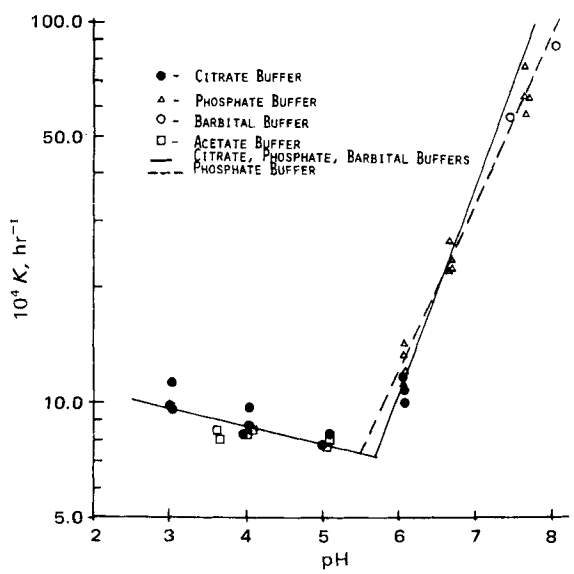

Figure 1—*Logarithm rate constant–pH profile at 278 °K.*

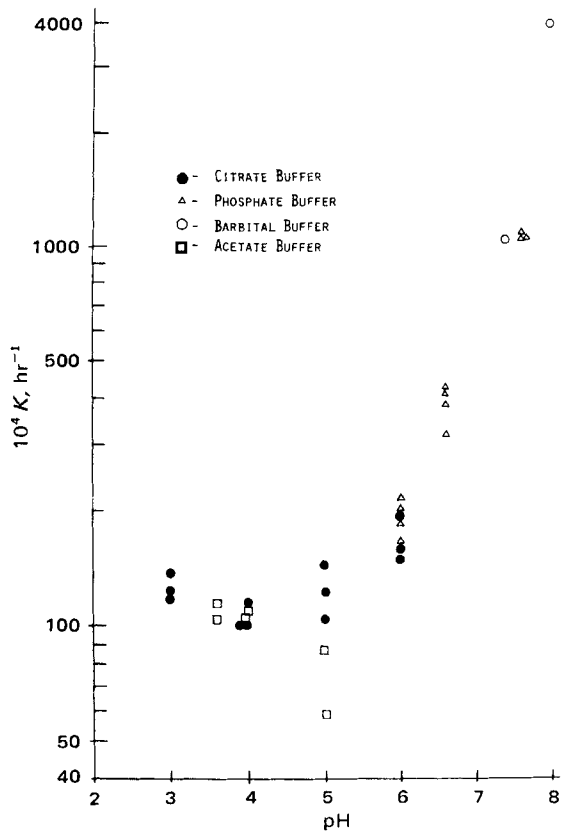

Figure 2—*Logarithm rate constant–pH profile at 295 °K.*

Examination of the linear regression lines for quadratic and cubic curvature revealed no significant departures from linearity for the data, indicating that the assumption of apparent first-order degradation is justified. When the rate constants for degradation in the various buffer solutions were plotted as the logarithm of the observed rate constants *versus* pH, the degradation rate minimum appeared between pH 5.2 and 5.5 (Fig. 1). The rate constants computed at a particular pH, regardless of the buffer system, remained similar, indicating the absence of catalytic effects by any one buffer species.

The results of regression of the logarithm of the degradation rate constants for I on pH in acetate and citrate buffers in the pH 3–5 range are summarized in Table I. The regression coefficient was nonzero and not equal to −1 at the 95% confidence level. Therefore, there is no reason to expect specific acid catalysis. Because the regression coefficient was significantly nonzero, the presence of only solvolysis was also ruled out and general acid catalysis was likely.

Determination of catalysis by base is also possible by examining Fig. 1 in the basic pH region.

The results of the regression of the logarithm of the apparent degradation rate constant for I on pH for citrate, phosphate, and barbital buffers in the pH 6–7.6 range appear in Table I and are illustrated in Figs. 1 and 2. The slope of the line was significantly nonzero but also significantly different than 1 at the 95% confidence level. This result indicates general base catalysis, which is disconcerting since the regression coefficient was nonzero and not significantly different than 1 at the 95% confidence level when only the phosphate buffers were used. The results are also shown in Table I.

The slope with combined citrate, phosphate, and barbital buffers increased with temperature, but the slope for the phosphate buffers showed no such tendency. If the degradation rate constant in a particular buffer was inordinately low at a low pH or high at a high pH, this result could be expected. The rate constants for I were lower in citrate buffer than in phosphate buffer at comparable pH values, and this difference increased with increasing temperature. While the difference at 5° was consistent but not great, the difference at 37° amounted to a factor of about 2. It is not likely that this result was anomalous, since it was consistent over all replications of both buffers.

[2] Bausch & Lomb Spectronic 20.

Table II—Effect of Total Phosphate Concentration on Apparent Degradation Rate Constant of I in Phosphate Buffer at Several pH Values

Ionic Strength	pH	Rate Constant, 10^4 hr^{-1} (*SE*) 5°	22°	37°
0.2	6.0	12.6 (0.30)	197.2 (3.11)	1281.5 (83.98)
	6.6	22.8 (0.45)	353.3 (24.20)	2818.4 (85.51)
	7.6	58.9 (2.82)	1069.0 (24.34)	5428.9 (549.29)
0.05	6.0	12.9 (0.22)	179.4 (24.89)	1395.2 (31.18)
	6.6	24.5 (0.45)	397.2 (11.48)	2922.4 (81.61)
	7.6	68.2 (1.17)	1053.3 (20.98)	7431.1 (384.5)

These data indicate that at pH 6 citrate seems to have a stabilizing effect on I. While the converse, that phosphate has a catalytic effect on nitrosourea degradation, was asserted (7, 18) and denied (4), the data in this experiment do not support the hypothesis of phosphate catalysis, primarily because the degradation of I in phosphate buffers was independent of phosphate concentration (Table II). Although the monohydrogen phosphate anion is said to be the catalytic species (7, 18), degradation rate determinations for I at differing total phosphate concentrations, and thus differing monohydrogen phosphate anion concentrations, provide a measure of such an effect. Since the rate constants at any particular pH and temperature with different ionic strength of phosphate buffers were the same within replication error, evidence for phosphate catalysis is lacking.

The observation that the slope of the logarithm of the apparent degradation rate constant *versus* pH for the phosphate buffers was not significantly different than 1 supports the view of no catalytic effect by phosphate. Finally, from examination of Figs. 1 and 2, it can be seen that the degradation rate constants for I in barbital buffer at pH 7.4 and for phosphate at pH 7.6 were similar, which suggests that no catalytic effect by phosphate or barbital was taking place. These observations tend to rule out the likelihood of specific phosphate catalysis for degradation of I.

The temperature dependence of the degradation rate constants for I in the various buffer systems provides data useful in performing accelerated stability analyses. The activation energies for the degradation of I in the various buffer systems were calculated by transforming the coefficient obtained for the regression of the logarithm of the apparent degradation rate constants on the reciprocal of absolute temperature (Table III). The activation energy appears to be proportional to the pH at which the activation energy was calculated.

To examine this possibility, the hypothesis that the activation energies are the same was tested using analysis of variance techniques (19) (Table IV); results indicated that this hypothesis should be rejected. To examine the possibility that there is a relationship between the activation energy and pH, a regression of the activation energy on pH was calculated (Table IV). While the regression of activation energy on pH was highly significant, the deviations from linear regression were also highly significant (Fig. 3). This relationship, however, seems linearized using a graph of the logarithm of the activation energy *versus* pH (Table IV and Fig. 4).

This effect of pH on the activation energy and linear relationship of the logarithm of the activation energy *versus* pH might be explicable if specific base catalysis is assumed. The rate constant used in determining the activation energy is the apparent rate constant or microscopic ex-

Table III—Activation Energies of I in Buffered Aqueous Media

Buffer	pH	Activation Energy, kcal/mole (*SE*)
Citrate	3.0	13.63 (1.56)
	4.0	15.40 (1.14)
	5.0	18.94 (1.43)
	6.0	20.17 (1.34)
Acetate	3.6	17.29 (1.98)
	4.0	19.88 (1.20)
	5.0	19.14 (0.74)
Phosphate (I^a = 0.2)	6.0	19.25 (2.02)
	6.6	25.55 (0.79)
	7.6	24.75 (0.81)
Phosphate (I = 0.05)	6.0	25.30 (0.37)
	6.6	25.95 (0.35)
	7.6	25.14 (0.39)
Barbital	7.4	25.92 (0.02)
	8.0	27.31 (0.12)

[a] I = ionic strength.

Table IV—Examination of Homogeneity of Slope of Activation Energy of I at Several pH Values and Regression [a] of Activation Energy on pH

Source of Variation	*df*	*SS*	*MS*
Homogeneity of Slope			
Among regression coefficients	9	6.8×10^5	7.6×10^4
Within regression (weighted mean of deviations)	14	2.4×10^2	1.7×10^1
Regression of Logarithm Activation Energy on pH			
Linear regression	1	0.525	0.525
Deviation from linear regression	8	0.069	0.008
Within pH groups	5	0.048	0.009

[a] The regression equation was ln $\Delta H^{\ddagger}$ (kcal/mole) = ln 10.51 + 0.12 (±0.016) pH.

perimental degradation rate constant, which includes contributions of all catalytic species. Since the macroscopic rate constant for the hydroxide anion has the greatest magnitude of the catalytic constants, the apparent degradation rate constant for which the activation energy is calculated is (20):

$$K_{app} = K_0 + K_{OH}[OH^-] \qquad \text{(Eq. 1)}$$

where K_{app}, K_0, and K_{OH} are the rate constants observed for the uncatalyzed reaction and for the hydroxide-ion contribution and $[OH^-]$ is the hydroxide-ion concentration.

The apparent activation energy computed from the slope of the graph is a function of the activation energy for K_0 as well as that for K_{OH}. Aside from demonstrating that there is a marked temperature dependence of the hydroxide-anion rate constant, the results indicate that the temperature dependence of the uncatalyzed solvolytic rate constant for I is not particularly strong.

The results indicate a minimum degradation rate between about pH 5.2 and 5.5. Examination of the degradation rate constants obtained in

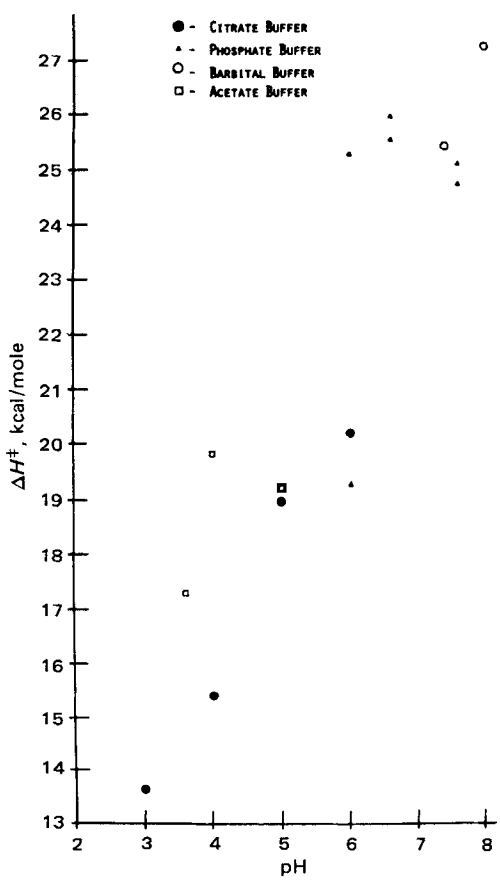

Figure 3—*Activation energy–pH profile for buffered aqueous systems.*

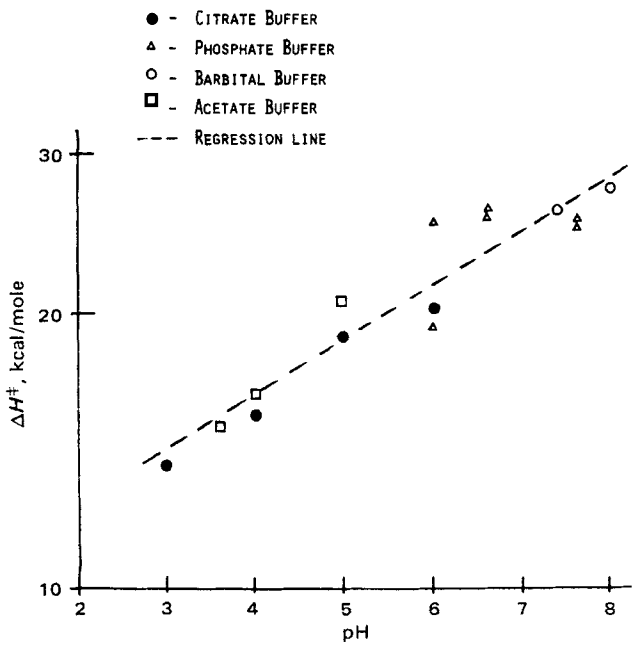

Figure 4—*Logarithm activation energy–pH profile for buffered aqueous systems.*

the accelerated stability analysis studies (Table I) indicates that I would have a short useful life at room temperature in the buffer systems studied. Ten percent degradation would occur in about 14 hr in the citrate buffer at pH 5.0. However, storage in the refrigerator (~5°) would prolong the time for 10% degradation to about 5.5 days.

These preliminary results indicate that it may be feasible to reconstitute I in some intravenous solutions at pH 5.2–5.5, to store the mixture at 5° for a few days, if necessary, and then to administer I parenterally. Further work is underway to determine the effect of certain electrolytes, commonly found in intravenous solutions, on the stability of I.

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Degradation of Carmustine in Mixed Solvent and Nonaqueous Media

PAUL A. LASKAR [x] and JAMES W. AYRES

Abstract □ The degradation rate of carmustine in several solvent mixtures and in mannitol solution was investigated at 5, 22, and 37°. The solvents chosen were those utilized as parenteral diluents. The apparent first-order degradation rate constants were computed using a linear regression procedure. The most nonaqueous solvent mixtures demonstrated minimum apparent degradation rates. The apparent degradation rate constant decreased with a decrease in the macroscopic dielectric constant. From the data at several temperatures, the apparent activation energies for carmustine degradation in the several solvent mixtures were calculated. There was no evidence for a relationship between the apparent activation energy and the dielectric constant.

Keyphrases □ Carmustine—degradation rate in mixed solvents and nonaqueous media, effect of temperature □ Degradation rate—carmustine in mixed solvents and nonaqueous media, effect of temperature □ Antineoplastic agents—carmustine, degradation rate in mixed solvents and nonaqueous media, effect of temperature □ Stability—carmustine in mixed solvents and nonaqueous media, effect of temperature

Numerous chemotherapeutic agents, including the nitrosoureas, show promise in the treatment of neoplastic diseases. The formulation of these agents into clinically useful dosage forms has, however, been slow. Carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea; NSC 409962] (I), like many other nitrosoureas, has relatively poor aqueous sol-